Peer Community In Microbiology

Addition of molybdate to shrimp ponds is a promising new technique to delay the accumulation of toxic H2S

Roey Angel *based on peer reviews by 2 anonymous reviewers*

Funda Torun, Barbara Hostins, Peter De Schryver, Nico Boon, Jo De Vrieze (2024) Molybdate delays sulphide formation in the sediment and transfer to the bulk liquid in a model shrimp pond. bioRxiv, ver. 3, peer-reviewed and recommended by Peer Community in Microbiology. https://doi.org/10.1101/2023.11.16.567380

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Shrimp aquaculture ponds are an established technology that helps answer the demand for high-protein food while reducing the impact of fishing on the oceans.

However, as a closed system, high in organic matter, aquaculture ponds in general and those used for shrimp in particular tend to develop anoxic sediments and favour sulfate reduction to H2S. The development of hydrogen sulphide, in return, is toxic to the shrimp and can lead to lower yields.

A standard solution to the problem is to inject air into the sediments. However, this solution requires additional infrastructure, is costly to operate, and can also disturb other essential life forms in the pond, such as benthic plants.

In this work by Torun et al. (2024), the authors used a carefully designed lab model of shrimp ponds to show that the addition of molybdate at concentrations as low as 5 mg/l delayed the accumulation of H2S and pushed the zone rich in sulphide deeper into the sediment.

The postulated mechanism for the inhibition in H2S production is that molybdate binds to the ATP sulfurylase in sulphate-reducing bacteria (SRB), and together with ATP, they generate adenosine 5'-phosphosulfate (APS) that cannot be used as an electron acceptor.

Surprisingly, however, the growth of SRB was stimulated rather than inhibited in this experiment. While the exact cause remains unknown, the authors postulate that SRB resorted to alternative metabolic pathways such as fermentation.

Overall, while this work was done on a model system in the lab, adding molybdate to shrimp aquaculture ponds is a promising technique and should be tested on a larger scale.

References:

Torun F, Hostins B, Schryver PD, Boon N, Vrieze JD. (2024). Molybdate delays sulphide formation in the sediment and transfer to the bulk liquid in a model shrimp pond. bioRxiv, ver.3, peer-reviewed and recommended by Peer Community In Microbiology.
https://doi.org/10.1101/2023.11.16.567380

Reviews

Evaluation round #2

DOI or URL of the preprint: https://doi.org/10.1101/2023.11.16.567380
Version of the preprint: 2

Authors' reply, 18 April 2024

Download author's reply
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Decision by Roey Angel, posted 08 April 2024, validated 09 April 2024

Decision on your revised manuscript: Long-term sulphide mitigation through molybdate at shrimp pond bottoms

Dear authors,

Thank you for submitting your revised manuscript entitled "Long-term sulphide mitigation through molybdate at shrimp pond bottoms" to PCI Microbiology.

I have now read your reply to the reviewers and your revised manuscript. I believe nearly all issues have been resolved, and the manuscript is now much improved.

However, one issue remains pending in my mind. Reviewer #2 asked whether the "ANOVA was preceded by a study of the underlying distribution of the data to determine if it was indeed normally distributed." From the manuscript and from your reply it is not entirely clear what exactly you modelled in this case. From your reply, it seems that you compared the relative abundance of each OTU across all the biological replicates of a given treatment to conclude if they differ. If this is the case, the procedure is termed "differential abundance testing" and is non-trivial. There is a vast literature on the subject and many methods. Briefly, however, ANOVA is not suitable for such data primarily because it is compositional in nature and not absolute (see 10.3389/fmicb.2017.02224).

ALDEX2 (10.1371/journal.pone.0067019) and ANCOM-BC2 (10.1038/s41467-020-17041-7) are two of the most popular methods for running such tests these days.

However, I would argue that there is no real reason to test the difference between the biological replicates. Instead, you should run a differential abundance test between your treatments or days to see which phyla changed (ANCOM-BC2 is particularly suitable for running tests on higher taxonomic levels). This is optional since I believe that the manuscript can be recommended even without such a test. However, the ANOVA test should be omitted.

Please feel free to contact me with any questions.
Sincerely,
Roey Angel

Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.1101/2023.11.16.567380
Version of the preprint: 1

Authors' reply, 28 March 2024

Download author's reply
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Decision by Roey Angel, posted 08 February 2024, validated 08 February 2024

Decision on your manuscript: Long-term sulphide mitigation through molybdate at shrimp pond bottoms

Dear authors,

Thank you for submitting your manuscript entitled "Long-term sulphide mitigation through molybdate at shrimp pond bottoms" to PCI Microbiology.

First, I would like to apologise for the longer-than-usual processing time of your manuscript. This delay stemmed mostly from the fact that many of our reviewers were on holiday in December and January.

Below, you will find the assessment from two expert reviewers. As you will see, both found your paper interesting, relevant, and scientifically robust but raised some questions and concerns. However, I believe that revising the manuscript according to the reviewers' suggestions should be rather quick, and I hope you will find their comments useful.

Your revised manuscript should be accompanied by point-by-point responses to the reviewers' comments.
Please feel free to contact me with any questions.
Sincerely,
Roey Angel

Reviewed by anonymous reviewer 1, 05 February 2024

The manuscript titled "Long-term Sulphide Mitigation through Molybdate at Shrimp Pond Bottoms" details the effects of molybdate addition (5 and 25 mg/L of Na2MoO4.2H2O) as a strategy to reduce H2S production by sulfate-reducing microbial communities in shrimp aquaculture ponds. While sulphide production was not completely prevented, molybdate addition delayed the onset of sulphide production and shifted the sulphide production zone to deeper sediment layers in the tanks. Unexpectedly, molybdate addition correlated with an increased abundance of putative sulfate-reducing populations affiliated with Desulfobacterota.

Overall, the study is interesting, the experimental rationale is solid, and the manuscript is well-written. However, I have one concern regarding the content and another regarding the form.

Concerning scientific content, I have a question about the various processes associated with the biogeochemical sulfur cycle in the ponds. It is noted that H2S concentrations, in the µM range, are lower in molybdate-treated ponds, while residual sulfate concentrations, in the 1000-1400 mg/L range, decrease similarly in both treated and untreated ponds. This raises questions about the different processes involved in the biogeochemical sulfur cycle in the ponds beyond sulfate reduction and their respective importance. H2S concentration results from various processes (sulfate reduction, sulfur oxidation, pH-dependent precipitation, liquid/gas equilibration phenomena, etc.). Although these are mentioned in the discussion section (lines 360-414), the manuscript's clarity and impact would be enhanced by explicitly stating and discussing these processes, perhaps through a diagram of hypothetical sulfur-associated processes with/without molybdate. This would also clarify the interpretation of results for non-specialized readers.

In terms of form, I find the manuscript clear and well-written, except for one main concern: the effect of molybdate addition and the operational applicability of the results seem overstated in the abstract and conclusion sections. While molybdate does affect the onset and localization of microbial sulfate-reducing activity in the sediments (e.g., Figure 3), its effect decreases over time and has limited impact on dissolved O2 and H2S concentrations in the bulk (Figures 1 and 2). The real benefit of the study lies in insights into the effect of molybdate addition rather than as a proposed operational mitigation strategy, which would require further studies and optimization. I recommend the authors remain more balanced regarding operational applicability in the abstract and conclusion sections and rephrase sentences such as:

- ”In conclusion, molybdate acted as a long-term mitigation strategy against sulphide accumulation by directly influencing the microbial community in a shrimp pond system.” (abstract)

- ”Overall, molybdate can serve as a more environmentally friendly option compared to other conventional strategies for mitigating sulphide production in shrimp pond systems.” (conclusions)

More specific comments are as follows:

Title:

The title suggests addressing the effect of molybdate in long-term experiments corresponding to the final stages of shrimp growth. However, ”Long term” as used in the title is misleading, as the mitigation appears to be temporary (see Figure 3) and its extent is limited (Figures 1 and 2). A rephrasing is suggested.

Abstract:

See the comment above.

Materials and Methods:

This section is clear and well-described. The clear description of the level of replication in each type of experiment and measurement is appreciated.

Results:

- Lines 253-255: The oxygen fluctuations affecting all pilots need clarification. Please mention possible causes or announce that possible cause are discussed latter in the discussion section.

- Lines 260-274: It is reported that H2S concentrations are lower in molybdate-treated ponds, while residual sulfate concentrations decrease similarly in both treated and untreated ponds (Table 2). This necessitates a discussion on the different types of processes associated with the biogeochemical sulfur cycle in the ponds for clearer interpretation of the results.

Discussion:

- Lines 378-390 and 403-414: The discussion is interesting, but it may not be fully understandable to a non-expert audience. A graphical scheme illustrating hypothetical sulfur-associated processes in the presence or absence of molybdate, distinguishing between processes supported by experimental data and those that are speculative, would aid the reader and enhance the manuscript’s scientific impact.

Reviewed by anonymous reviewer 2, 08 January 2024

I was pleased to have the opportunity to review this interesting and workmanlike manuscipt. The authors present the results of a carefully designed study, the results of which have been thoughtfully presented and discussed with clarity and modesty.

In essence adding molybdate works in that it suppresses sulphide production but, for some reason the additive did not suppress the sulphate reducing bacteria (SRB).

The careful design included excellent replication of the sort that is most easily executed in a model system. However, it is not clear from the title that a model system has been used. I wonder if the authors would consider using the phrase “model shrimp pond”. If only to telegraph to the reader that this is a study of a model and not a real system.

As noted above, the use of a model allowed high levels of control and replication. However, it may also have meant no sunlight, this in turn might affect photosynthesis and thus pH and oxygen. I assume algae normally grow in shrimp ponds but were absent from the model system. Would the authors care to comment on this? pH is important to sulphide toxicity and high pH might offset elevated sulphide levels.

The study itself was conducted over 60 days which I understand is the period it takes to grow and harvest shrimp. However, I am not sure if this is a “long term” study. However, this is a minor point and to some extent dependent on ones personal point of view.

The methods are very well described and I commend the authors for the use of flow cytometry to get some actual numbers, as well as the high levels or replication. However, I did wonder if use of ANOVA was preceded by a study of the underlying distribution of the data to determine if it was indeed normally distributed.

The results are nicely presented, and the oxygen and sulphide profiles are fascinating. It is not always easy to compare the different test conditions as they are in different panels of the same graph. Have the authors considered comparing the sulphide profiles for experiment on a given day on the same graph? It might be no improvement, but if it was possible the differences between experiments would be clearer.

The discussion is sensible and to the point and contains excellent suggestions as to the underlying cause of the strange increase in SRB. I did wonder if the increased diversity might also be associated with an increase in evenness that in turn would permit more SRB to be detected. However, suspect this is a minor point give the finding that the number of SRB increased.

However, given that this is a model study, it might be worth adding a sentence or two about the implications of using molybdate at full scale. For example, how much molydate might be required and what tradeoffs might need to be considered. However, any discussion should not be at the expense of the admirable brevity of the present discussion.